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REVIEW

Translating St m and Progenitor Cell Biology to the Clinic: Barriers and Opportunities

Irving L. Weissman

Stem cells are the natural units of embryonic generation, and also adult regeneration, of a variety of tissues. Recently, the list of tissues that use the model of differentiation from stem to progenitor to mature cell has increased from blood to include a variety of tissues, including both central and peripheral nervous systems and skeletal muscle; it is also possible that all organs and tissues are derived from, and still contain, stem cells. Because the number and activities of stem cells and their progeny are homeostatically regulated, clinical stem cell transplantation could greatly add to the physician's armamentarium against degenerative diseases.

The clinical application of stem and progenitor cell transplantation began with the exposure of civilian populations to lethal doses of radiation in 1945. Irradiation of mice replicated the syndrome, and bone marrow (BM) transplants radioprotected them by providing donor-derived hematopoiesis (*1–3*). In 1961, Till and McCulloch demonstrated the existence of clonogenic BM precursors that give rise to multilineage hematopoietic colonies in the spleen [colony-forming units, spleen (CFU-S)]; a subset of spleen colonies contained cells capable of forming more spleen colonies. They proposed that these were pluripotent hematopoietic stem cells (HSCs) (*4–6*) that had the property, at the single-cell level, of (i) self-renewal as well as (ii) multilineage differentiation. This remains the enduring definition of stem cells.

Whereas the above-described experiments provided evidence that stem cells exist, they did not enable their isolation. With the development of quantitative assays for clonogenic precursors in mice of all hematolymphoid precursors (*7–9*), a reductionist approach was developed for the identification and isolation of HSCs. Monoclonal antibodies (mAb) were identified that bind cell surface markers on some, but not all hematopoietic cells; marker positive and negative subsets were separated by cell sorting (e.g., fluorescence-activated cell sorting) to identify cells with clonogenic precursor activity (*9*). Eventually, clonogenic multipotent progenitors with a distinctive marker profile proved to be HSCs (Fig. 1A) (*10–13*). A subset of this population perpetually self renews; these are long-term stem cells (LT-HSCs) (*12, 14*). All subsets of these HSCs were radioprotective, and HSCs were the only radioprotective elements in mouse bone marrow (*11*). As the HSC cell dose increased, the time to engraftment of clinically protective numbers of donor-derived blood cells shortened (Fig. 1B) (*15*). Subsequently, in vitro and in vivo assays for clonogenic human stem and progenitor hematolymphoid cells were developed and by the same approach candidate human HSCs were isolated (*16, 17*).

The mouse and human HSCs depicted in Fig. 1A were the first isolated by surface markers. It was subsequently shown that both $CD34^+$ and very rare $CD34^-$ subsets of LT-HSCs exist (*18, 19*); HSCs actively extrude dyes such as Hoechst 33324 and Rhodamine 123, and can be isolated by this property (*20*). In humans, the mAb AC133 also identifies HSC (*21*).

Purified human HSCs are capable of hematopoietic reconstitution in patients receiving bone marrow ablative (myeloablative) doses of radiation and chemotherapy. Increasing the dose of HSC shortens the time to engraftment of mature blood elements in man as in mice (Fig. 1C) (*22–24*).

Biology of Hematopoietic Stem and Progenitor Cells

In mice, LT-HSCs give rise to short-term HSCs (ST-HSCs), which give rise to multipotent progenitors (MPPs), whose further progeny are oligolineage-restricted (Fig. 2) (*12*); dedifferentiation cannot be detected (*25*). HSCs can first be found in the developing yolk sac blood islands; transfer of blood island cells to same age-hosts resulted in lifelong, donor-derived hematopoiesis (*26*). HSCs can also be found in the embryo proper (*27, 28*). HSC are next found in the fetal liver (*13*), and then the fetal spleen and BM (*29*); each stage occurs presumably by HSCs entering the fetal circulation. In young adult mice, about 8% of the LT-HSC population randomly enters cell division per day, and on average, half their progeny must be LT-HSCs to maintain the steady-state level. As HSCs progress to MPPs, the frequency of cells in cycle increases (*30, 31*). In very old mice, most LT-HSCs are in cycle (*32*).

Dividing HSCs have four developmental choices: self-renewal, differentiation, programmed cell death, and emigration (*33*). The frequency of HSCs in hematopoietic organs is regulated by the fraction of stem cells that choose one or another of these fates. Transgenic expression of the anti-programmed cell death gene *bcl-2* (a proto-oncogene) in HSC results in an increase in their frequency in BM (*34*). These HSC have increased chemo- and radiotherapy resistance, a property that would be much valued clinically if *bcl-2* expression could be regulated.

The movement of stem cells between primary hematopoietic sites occurs naturally throughout life (*35*). Clinical provision of cytokines such as G-CSF alone or along with cytoreductive drugs [for review, see (*36*)] can induce mobilization of stem cells to blood (MPB), where they are collected for transplantation. Natural and induced HSC mobilization begins with mitotic expansion of HSC, followed by the release of G_1 HSC to blood to seed secondary sites (*35*).

Oligopotent progenitors downstream of HSCs have also been isolated (Fig. 2) (*37, 38*); HSC give rise alternatively to the clonal common lymphocyte progenitor (CLP), or the clonogenic common myeloid progenitor (CMP). CMP, in turn, can give rise to megakaryocyte or erythrocyte progenitors (MEPs), or granulocyte/monocyte progenitors (GMPs). None of these progenitors dedifferentiate or show self-renewal capacity (*37, 38*).

Broadening the Stem and Progenitor Cell Concept to Other Tissues

In vertebrates, the zygote is a totipotent stem cell, as are virtually all of its progeny around the blastula stage; cells contained within the inner cell mass (ICM), include (and may be composed of) totipotent stem cells (TSCs) (Fig. 3) (*39*). Embryonic stem (ES) cells are derived from cultures of ICM cells, and have the property of participating as totipotent cells when placed into host blastocysts. The developmental pathways that endogenous ICM cells or transferred ES cells take to tissue formation and organogenesis has led many to hope that these pathways can be controlled for the development of tissue and organ specific stem cells (*40*). However, we currently have an insufficient understanding of the developmental events that lead to organogenesis from ICM cells to program the production of tissue- or organ-specific stem cells.

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During vertebrate development, at defined stages the derivatives of the embryonic germ layers of endoderm, ectoderm, and mesoderm are involved in tissue formation and organogenesis. What is not yet clear is whether every tissue uses the stem and progenitor model shown to be operative in hematopoiesis (Fig. 3). It is reasonable to propose that most, if not all tissue and organ systems are based on a stem and progenitor model during organogenesis and that stem cells are retained throughout life to participate in regeneration and repair. If this thesis is correct, it would follow that the lessons learned from regeneration and repair of the hematopoietic system might be useful for the regeneration and repair of other organ systems.

The value of using the body's own stem and progenitor cell plan of tissue and organ regeneration is that their numbers and fates are regulated. For example, one cannot deliver too many HSC; regeneration derived from these stem cells results in regulated hematopoiesis. The advantages of a medicine based on stem or progenitor cell transplantation are (i) that one need not understand the process in detail to apply the therapy, (ii) that the applied therapy should have attendant toxicities only during the acute phase of host preparation for stem or progenitor transplants, and (iii) that the therapy is applied just once. In contrast, medical therapies based on substances that affect endogenous molecular targets will usually have effects and toxicities wherever the molecule is expressed; such therapies are by their nature chronic and are required for the duration of the disease.

Rat neural crest stem cells have also been isolated (*41*). Using as an assay the clonogenic reconstitution of in vitro multilineage neural cultures, we have enriched for candidate human fetal brain CNS stem cells (CNS-SCs) (*42*). The existence of CNS-SCs had been shown by retrovirus marking of cells (*43*). Transplantation of clonally-marked cells gave rise to neurons and glia whose cell fates were dictated by the regional CNS microenvironment (*44*). Continuing neurogenesis can occur in the adult brain in particular microenvironments such as the dentate gyrus and the subventricular zone (*45*). Candidate CNS-SCs at the single-cell level can produce neurospheres of multiple neural cell types; expanded numbers of cells in neurospheres can be prospectively isolated and are clonogenic precursors of neurospheres (*42*). These neurosphere cells can be transplanted into immunodeficient newborn mice or immunosuppressed adult rats and participate in neurogenesis of neurons and glia.

In skeletal myogenesis, current leading stem cell candidates are the satellite cells (*46*). Enrichment of precursors for blood vessels (*47*) and for skin (*48*) has been accomplished. Several unusual outcomes of cell transplantation have been reported: these include blood derived from clonal neurosphere cultures (*49*), blood derived from myogenic precursors (likely satellite cells) (*50*), myogenesis and vasculogenesis from isolated blood and bone marrow precursors (*51, 52*), and even participation of hematopoietic cells in neurogenesis (*53*) or liver development (*54*). It is not clear how this happens. For purposes of this review, the means by which organ-specific stem cells seem to change fate are relevant only to the extent that such cells are potential sources of expanding cells for transplantation (*55, 56*).

Clinical Transplantation of Stem and Progenitor Cells: Current Practice, Barriers to Their Accomplishment, and Opportunities

Hematopoiesis as a model of stem and progenitor transplantation. BM transplantation was invented to enable physicians to increase chemotherapy and radiotherapy to myeloblative doses with the objective of eliminating endogenous cancer cells. The first transplants that were successful were between identical twins, wherein no histocompatibility barrier of host against donor, and no opportunity of immune based reactivity of donor against host, exists (*57*).

Autologous BM or MPB transplants. Autologous hematopoietic transplants have been used in many patients with cancers, including those of the hematolymphoid system (lymphomas and leukemias), of plasma cells (multiple myeloma), and breast cancer (*58*). But even if these tumors are sensitive to chemotherapy, only a fraction of patients are cured. Why is this? First, in many patients, disease recurs at the primary site; thus, in many patients the level of therapy did not eliminate endogenous tumor. Second, the bone marrow and the MPB of patients with these cancers are often contaminated with cancer cells (*59*). Without elimination of these potentially clonogenic cancer cells, it

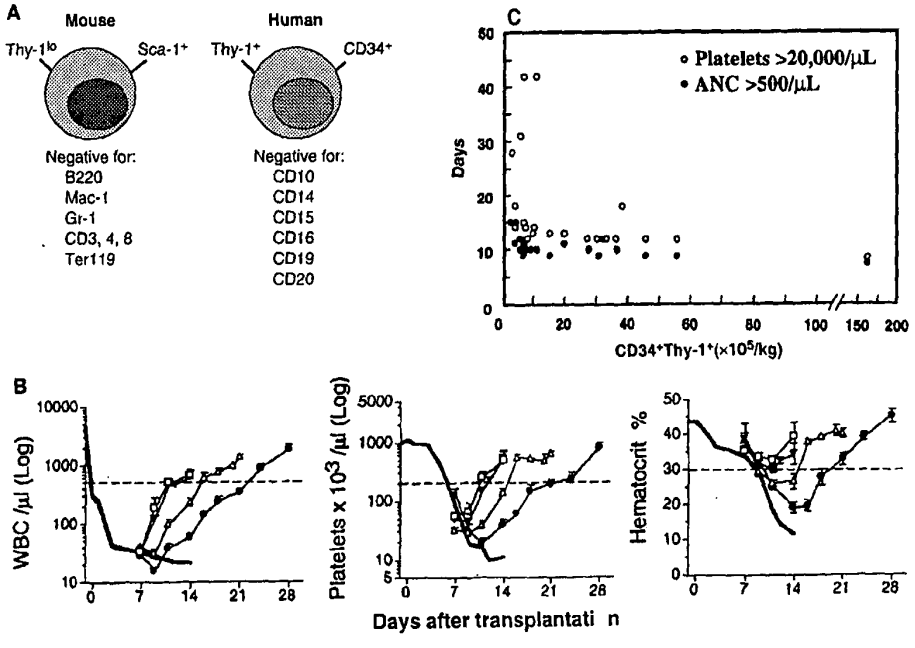

Fig. 1. (**A**) Clonogenic multipotent progenitors have a distinctive marker profile. Shown are surface phenotypes of HSCs in human and mouse. (**B**) Hematopoietic recovery in lethally irradiated C57BL/Ka mice syngeneically transplanted with different doses of purified HSCs. Irradiated mice were injected with 100 (solid circles), 1000 (open triangles), 5000 (solid triangles), or 10,000 (open squares) HSCs. Shown are the recovery kinetics for white blood cell (WBC), platelet, and hematocrit counts. The dashed horizontal line represents recovery of blood levels to 500 WBCs/μl, 200,000 platelets/μl, and 30% hematocrit (*15*). (**C**) Transplantation of highly purified human HSCs in patients with metastatic breast cancer. Shown are the times to engraftment of neutrophils [absolute neutrophil count (ANC) >500/μl] following transplantation with purified $CD34^+Thy1\text{-}1^+$ hematopoietic stem cells (*22*).

stands to reason that the benefits of high dose chemotherapy could be countermanded by the reintroduction of malignant cells to the circulation. Isolation of human $CD34^+Thy^+$ HSC from MPB can result in the elimination of detectable malignant multiple myeloma (*23, 60*), breast cancer (*22*), and lymphoma cells from the transplant (*24*). In the trial in Fig. 1C, the number of transplanted malignant cells was undetectable; further clinical trials with human HSC and MPB seem warranted. [I would like to warn the reader that I was co-founder of the company (SyStemix, Inc.) that initiated and carried out the trials, and therefore I might have biases]. It is likely that widespread cures of malignant disease by HSC transplants will not occur unless patients are subscribed earlier in the course of their disease, or if therapies adjuvant to the transplants are attempted. One direction of adjuvant therapy that can eventually be applied will be to attempt to regenerate or reconstitute specific immune responsivity to the small amount of residual tumor. For many tumors, immunity can be induced and is mainly T cell–based. T cell immunity can detect tumor-unique antigens or tumor-associated peptides that are derived from proteins specific to differentiating cells and are presented on the cell surface by self human lymphocyte antigen (self HLA) molecules (*61*). The antigen specific T cell receptors that recognize, for example, HLA-A2 and the enclosed melanoma peptide MAGE are entities that retain their specificity no matter whose T cell expresses them, opening the possibility of T cell receptor (TCR) gene transfection to endow antitumor immunity. The collection of T cells that recognize a particular HLA plus tumor peptide can be detected and isolated by a new technology of producing fluorescent major histocompatibility complex (MHC) peptide tetramers (*62*). Perhaps TCR transfection of HSC/CLP/T cells and/or tetramer-based T cell isolation will enable transplantation of the specific component of immune reconstitution in patients with minimal residual disease following HSC transplant. Additional strategies to augment these immune cell therapies include vaccination with gene-altered tumor cells (*63*), or augmenting and prolonging the antitumor T cell response by preventing their shutdown (*64*).

Allogeneic Transplantation of Hematopoietic Cells

Allogeneic hematopoietic grafts are potentially useful in cancer treatment, as they are not contaminated with cancer cells; unfortunately, BM and MPB contain T lymphocytes (*58, 65*). These donor T cells encounter and respond to host antigens in virtually all tissues in the body, leading to a multisystem graft-versus-host (GvH) syndrome (*58*). HLA-mismatched hematopoietic grafts are usually rejected (*66*). The high degree of HLA polymorphism makes a random match between unrelated humans a rare event (*58*). The probability of an HLA match is 25% between siblings. Because MHC molecules process and present any of a number of peptides present within a cell, siblings that share HLA may not share all tissue-specific peptides; these peptides create minor histocompatibility antigens when presented by shared HLA molecules. Minor histocompatibility antigens are important for both host rejection of grafts and GvH immunity (*67*). HLA-matched host-versus-graft and GvH immunity can largely be controlled by highly immunosuppressive treatments that have attendant risks of chronic immunosuppression (*68, 69*). Patients given limiting numbers of hematopoietic cells often fail to engraft if T cells are eliminated, but engraft (and get GvH disease) if donor T cells are retained (*65*). These T cells are said to "facilitate" engraftment (*70–73*). The presence of facilitator cells raises the hope that one can cotransplant these cells with HSC to facilitate engraftment without GvH (*70–73*). However, in mouse models simply raising the HSC dose is sufficient to guarantee rapid and sustained engraftment in the absence of either failure to engraft or GvH, even if the mice are full H-2 mismatches (*15, 74*). For patients without cancer that require allogeneic hematopoietic or HSC transplants, it would appear that HSC alone at high doses would be most useful. In mouse studies, HSC doses sufficient to obtain rapid engraftment in the autologous setting are also doses sufficient to provide engraftment in the fully allogeneic, MHC mismatched setting (*15*).

In the case of HLA-matched allotransplants for leukemia, T cells can carry out a graft-versus-leukemia (GvL) response (*75*). Contained within the population of GvH T cells are cells that can recognize tissue-specific peptides in the context of shared HLA (*76*). Clinicians have fine tuned this response to the extent that initial hematopoietic cell grafts can be followed by donor lymphocyte infusions (DLI), when the patient is much healthier and when the patient's GvH response has been controlled. A significant fraction of patients with chronic myelogenous leukemia are in prolonged or complete remission as a result of DLI (*77, 78*). Recently, "mini-transplants" of HLA-matched MPB into sublethally treated hosts receiving drugs more specific for T cell immunity are followed by DLI; this process provides a lower transplant associated mortality and morbidity, retaining the benefits of GvL (*79*).

Allogeneic HSC and progenitor transplants can be used in a nonmalignant setting to restore the hematolymphoid system of the host (*80*). For example, a number of monogenetic disorders lead to deficiencies in cells within the hematolymphoid system, including a variety of severe combined immunodeficiencies and hemoglobin disorders (*65*). Repair of the defective enzymes or defective globins could come about either by allogeneic HSC transplants, or autologous gene corrected HSC transplants. The application of transgenic corrections of hematopoietic stem cells has been slowed by problems at a number of stages, but many of these problems have been solved [for example, (*81*)].

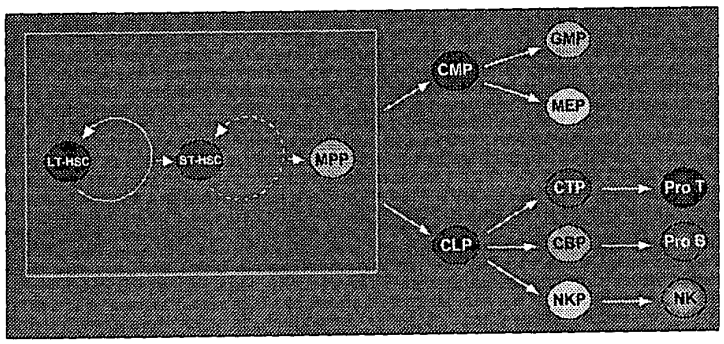

Fig. 2. Model of HSC differentiation. Included in the progeny of mouse HSCs are two kinds of oligolineage restricted cells: common lymphocyte progenitors (CLPs) (*37*), that at the clonal level are restricted to give rise to T lymphocytes, B lymphocytes, and natural killer (NK) cells; and CMPs (*38*), which are progenitors for the myeloerythroid lineages. Single CMPs give rise to myelomonocytic progenitors (GMPs) and megakaryotic/erythroid progenitors (MEPs). All of these populations are separable as pure populations, using cell-surface markers (*38*).

The Use of Allogeneic HSCs for the Induction of Specific Lifelong Transplantation Tolerance

It has been known since the late 1950s that allogeneic bone marrow hematopoietic grafts into irradiated hosts can lead to donor specific chimerism for the life of the host [reviewed in (*82*)]. These hosts have hematolymphoid systems that are derived wholly or in part from donor stem cells. Such hosts are usually permanently tolerant of donor organ or tissue transplants. Thus, one can transplant fully

allogeneic HSC and cotransplant, for example, hearts from the HSC donors, and produce specific and lifelong acceptance of the transplant, with retention of reactivity to third party grafts and pathogens [reviewed in (*80*)]. Cotransplantation of HSC and stem cells for other tissues or organs from the same donor ought to be possible, and ought to enable a circumstance wherein sublethal conditioning of the host permits hematolymphoid chimerism for the purpose of tolerance induction, and cell- and organ-specific regeneration for the replacement of diseased or destroyed organ systems.

Allogeneic HSC Transplants for MHC-Determined Autoimmunity

Many of the autoimmunities are genetically based, especially those that involve an autoimmune response of T cells to organ- or tissue-specific antigens, such as in type 1 diabetes (the insulin producing islets are their principal target) (*83*) and multiple sclerosis (the myelinated nerve sheaths are targets). In these cases, the predilection for development of autoimmune T cells can map to particular MHC alleles (*84*). In mice, HSC transplants from normal donors into lymphoablated diabetogenic (NOD) hosts can abrogate an ongoing diabetogenic autoimmune T cell response (*80*). The hosts are tolerant of subsequently transplanted donor strain islet grafts (*85*). Thus, allogeneic HSC transplants can abrogate autoimmunity and induce transplantation tolerance for subsequent stem cell, tissue, or organ grafts.

Transplantation of Nonhematopoietic Stem Cells

The aforementioned models provide a means by which tolerance can be induced to a particular donor set of transplantation antigens. In the case of patients with diseases wherein the generation of mature or maturing cells of a particular organ system is a central problem, cotransplantation of HSCs and nonhematopoietic stem cells should enable organ regeneration.

The recent identification of candidate CNS-SCs and the ability to grow them to large numbers in in vitro cultures should allow testing of the notion that such cells would be capable of regenerating neural or glial elements when necessary (*56*). Transplantation of tissues that include dopaminergic neurons such as adrenal medulla, fetal ventral mesencephalon, and teratomas are currently being tested in animal models and human cases of Parkinson's disease (*45*). Rodent CNS cell lines that include CNS-SC, sometimes immortalized with v-myc (*86*), have been used in a number of models of mouse genetic neurodegenerative diseases, including demyelinating diseases (*87*), brain gangliosidosis, and other neurodegenerative disorders (*88*). It is not clear which is the appropriate cell to transplant—the CNS-SCs, the required neurons, or the intermediate progenitors between the two. In the hematopoietic system only HSC are required (*11, 15*). Although one might think that in the nervous system more differentiated neurons are the appropriate transplants, the normal generation and regeneration of different parts of the brain occurs via stem cells, and it is conceivable that only stem and progenitor cells have both the migratory capacity and the differentiation pathways capable of treatment of these neural defects. Thus, in neurodegenerative diseases, it is important first to determine the rules of transplantation of stem, progenitor, and mature cells, as well as determine the sites into which the transplants must be placed.

Other potential neurological disease targets include multiple sclerosis, where an ongoing T cell response might be abrogated by allogeneic HSC transplants or other potent immunosuppressive maneuvers (*89*). In these cases, remyelination from endogenous precursors is not guaranteed, and the use of neurogenic stem cells, their oligopotent progeny, the immediate precursors of myelinating glia, or the glia cells might provide tissue specific remyelination and regeneration of function. Other potential targets of neural and progenitor cell transplants might include tissues damaged by small strokes, spinal cord injuries, etc.

Transplantation of Other Stem or Progenitor Cells

Liver organ transplants are the therapy of choice in a number of conditions wherein the liver is damaged by toxins, drugs, viral infection, or if the patients have gene defects in the production of important liver-generated factors or receptors. For the most part, liver transplants require a recently deceased but still perfusing donor, and long waiting lists exist for liver transplants. Of course, because donors who recently died are most likely not HLA-matched to the recipient, liver transplants are usually HLA-disparate and require powerful immunosuppression. It is reasonable to assume that if liver-repopulating stem or progenitor cells are available, sibling transplants may become feasible.

The identification of islet stem and progenitor cell populations appears to be at an earlier phase of development (*90*). Islet cell transplantation would be preferable to multiple insulin treatments daily, as these are the cells that both sense the circulating levels of glucose and respond appropriately by releasing insulin at the right dose and tempo. The complications of diabetes are frequent, life-

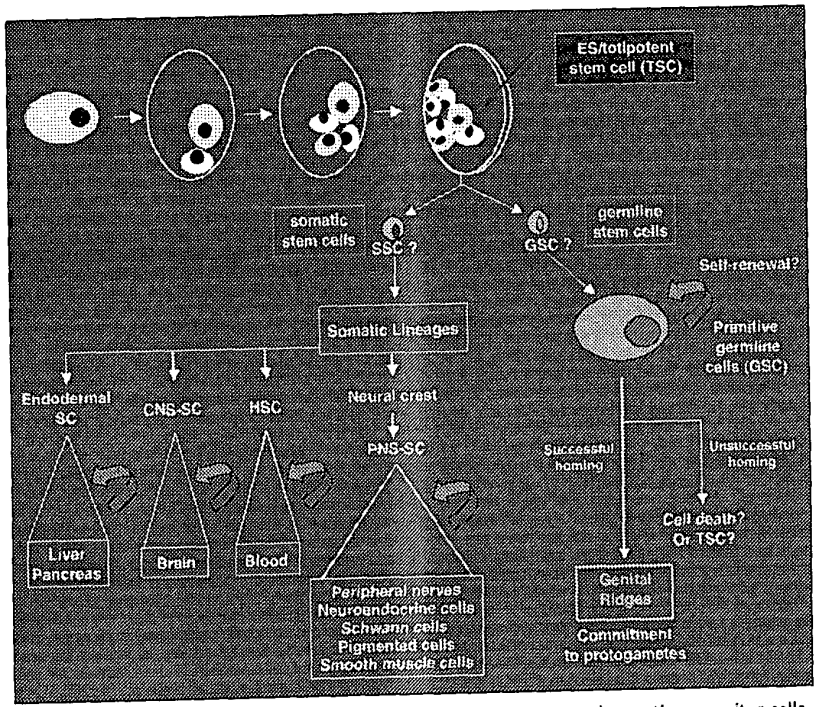

Fig. 3. Model of totipotent stem cell (TSC) generation of germ line and somatic progenitor cells. TSCs can diverge into primitive germ line cells (GSCs) and to tissue-specific stem cells, perhaps through a putative common somatic stem cell (SSC). The organ- and tissue-specific stem cells on the left are populations of cells we propose are the primary cells that are or will be used in clinical transplantation. On the right side of the figure are populations of TSCs that could eventually be useful as precursors of organ- and tissue-specific stem cells.

shortening, and difficult to manage by insulin therapy. Whole pancreas transplants are difficult, as in liver transplantation. Islet transplants require large numbers of viable cells, and as yet islet cells are difficult to expand in vitro. Thus, it is a reasonable goal to search for conditions wherein islets are continuously generated from stem/progenitor cells, as in some mouse models (*90*), and to replicate them in vitro.

Muscle regeneration in the case of the intrinsic muscular dystrophies or muscle loss conditions could be life-saving. The recent isolation of skeletal muscle satellite stem cells (*46*) gives hope that stem cell therapy can be applied to these conditions. Another frequent target for muscle regeneration is the heart, where rapid cell death following coronary artery blockage is a major cause of mortality and morbidity. Unfortunately, the satellite cell equivalent in the heart tissue has not yet been found.

It is reasonable to expect that cotransplantation of HSCs and tissue or organ stem and progenitor cells will occur increasingly over the next two decades and will result from the intersecting advances in stem cell biology and stem/tissue transplant immunology.

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